

K⁺ current expression and proliferation of human T-lymphocytes induced by various stimuli

Pethő Zoltan Denes, Balajthy Andras

Department of Biophysics and Cell Biology, Debrecen Medical and Health Science Centre,
University of Debrecen
98, Nagyerdei krt., Debrecen, Hungary, H-4012

pethozo@freemail.hu

Ion currents of human T-lymphocytes and specifically Kv1.3 potassium channel currents are crucially important for the triggering of an immune response. Resting T cells also express this channel, but as a result of stimulation (e.g. an immune reaction), the expression of the channel increases. In contrast to the *in vivo* activation of T cells, where the interaction with specialized antigen presenting cells triggers T cell proliferation, *in vitro* stimulation of the cells uses molecules that can act on specific signal pathways. Our aim was to investigate whether Kv1.3 channel expression in T-cells depends on the nature of the signaling pathway activated *in vitro*.

We used healthy human peripheral blood T-cells in our experiments. We monitored the division of the cells in a flow cytometer using CFSE (carboxyfluorescein succinimidyl ester) dilution assay. The cells were stimulated with a mitogenic lectin (phytohaemagglutinin: PHA); a combination of a diacylglycerol analog (phorbol 12-myristate 13-acetate: PMA) and a calcium ionophore (ionomycin); furthermore with antibodies affecting the TCR-CD3 complex (anti-CD3 by itself; anti-CD3 and anti-CD28 used in combination). Five days after application of the stimulus we measured the proportion of dividing cells, change in cell size and cellular granulation. Subsequently we measured the Kv1.3 ion currents through the T-cell membrane as well as biophysical parameters of this channel (e.g. activation and inactivation kinetics and voltage dependence of conductance), using the whole-cell patch-clamp technique.

Our flow cytometric measurements indicated that as the cells divided, cell size and amount of granulation increased after all treatments. Our patch-clamp experiments showed that there are significant changes in Kv1.3 ion currents at +50 mV depolarization after both PHA and anti-CD3+anti-CD28 stimulation. Inactivation kinetics changed only as a result of anti-CD3+anti-CD28, which could be a sign of posttranslational modulation of the channel. Similarly, increase in the current density was only significant in case of anti-CD3+anti-CD28 treatment. We can conclude that for measurement of Kv1.3 currents (e.g. for ion channel pharmacology), PHA treatment is sufficient but for the simulation of *in vivo* T-cell activation the most effective method is stimulation with anti-CD3+anti-CD28.